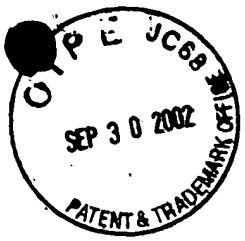




## APPENDIX A

Pursuant to 37 C.F.R. §1.121(c)(1)(ii), Applicant presents herewith marked-up text of the claims of this application as amended by the foregoing amendment.

1. (Twice Amended) A method of **[providing]** **preparing** a composition comprising a mixture of cells derived from human liver tissue, which mixture comprises an enriched population of human liver progenitors, the method comprising:

(a) providing a **[substantially single]** cell suspension of human liver tissue comprising a mixture of cells of varying sizes, including immature cells and mature cells; **and**

(b) debulking the suspension **based on cell size, buoyant density, or a combination thereof to remove mature cells** **[under conditions that permit the removal of mature cells and those of relatively large size], while retaining immature cells [and those of relatively small size: and],**

**[(c) selecting those cells which themselves, their progeny, or more mature forms thereof exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both,]**

to provide a mixture of cells comprised of an enriched population of human liver progenitors.

7. (Canceled)

8. (Amended) The method of claim 1 in which the debulking step comprises centrifugal elutriation, density gradient centrifugation, **[panning, affinity chromatography, tagging with fluorescent labels,]** countercurrent fluid flow, continuous-flow centrifugation, zonal centrifugation, **[use of magnetic beads,]** or combinations thereof.

11. (Amended) A human **[liver]** **hepatic pluripotent** progenitor isolated by the method of claim 1.

12. (Twice Amended) A method of **[providing]** **preparing** a composition comprising an enriched population of human **[liver]** **hepatic** progenitors comprising:

(a) providing a **[substantially single]** cell suspension of human liver tissue, **[and]**

**(b) debulking the suspension based on cell size, buoyant density, or a combination thereof to remove mature cells, and**

**(c)[(b)]**subjecting the **debulked** suspension to a positive or negative immunoselection, such that a mixture of cells is provided, which mixture of cells is comprised of an enriched population of human liver progenitors, which human liver progenitors themselves, their progeny, or more mature forms thereof exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both.

20. (Amended) A human **[liver]** **hepatic pluripotent** progenitor isolated by the method of claim 14.

21. (Twice Amended) A composition comprising an enriched population of human **[liver]** **hepatic pluripotent** progenitors, their progeny, or more mature forms thereof, which exhibit one or more markers indicative of expression of **full-length** alpha-fetoprotein, **full-length** albumin, or both.

27. (Amended) A method of treating liver dysfunction or disease responsive to treatment with liver progenitors **[in a subject in need thereof]**, comprising administering to **[the]** **a** subject **in need of such treatment** an effective amount of human liver progenitors, their progeny, more mature forms thereof, or combinations thereof, in a pharmaceutically acceptable carrier and treating the liver dysfunction or disease.

35. (Amended) A method of treating a disease **[in a subject in need thereof]** comprising administering **to a subject in need of such treatment** an effective amount of human

hepatic progenitors, their progeny, or more mature forms thereof in which the human hepatic progenitors, their progeny, or more mature forms harbor exogenous nucleic acid.

42. (Twice Amended) Isolated human **[liver]** **hepatic pluripotent** progenitors, their progeny or more mature forms thereof which exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both.

43. (Twice Amended) Isolated human **[liver]** **hepatic pluripotent** progenitors, their progeny or more mature forms thereof which exhibit the phenotype glycophorin $A^-$, $CD45^-$, alpha-$fetoprotein^{+++}$, $albumin^+$, and $ICAM^+$.

45. (Amended) The method of claim **[3]** **1** in which the **[immature cells]** **progenitors** have a diameter **[greater than about]** **between** 5 **and 15** microns.

46. (Amended) The method of claim **[3]** **45** in which the **[immature cells]** **progenitors** have a diameter **[greater than about]** **between** 8 **and 9.4** microns.

**47. (New) The method of claim 1 which further comprises selecting those cells which their progeny, or more mature forms thereof exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both.**

**48. (New) The method of claim 47 in which the selection step comprises panning, affinity chromatography, tagging with fluorescent labels, use of magnetic beads, or combinations thereof.**

## APPENDIX B

For the convenience of the Examiner, Applicant presents herewith a copy of the claims that will be pending upon entry of the present amendments.

1. (Twice Amended) A method of preparing a composition comprising a mixture of cells derived from human liver tissue, which mixture comprises an enriched population of human liver progenitors, the method comprising:

(a) providing a cell suspension of human liver tissue comprising a mixture of cells of varying sizes, including immature cells and mature cells; and

(b) debulking the suspension based on cell size, buoyant density, or a combination thereof to remove mature cells, while retaining immature cells,

to provide a mixture of cells comprised of an enriched population of human liver progenitors.

2. The method of claim 1 in which the liver tissue is obtained from a fetus, a neonate, an infant, a child, a juvenile, or an adult.

3. The method of claim 1 in which the immature cells have a diameter less than about 15 microns.

4. The method of claim 1 in which the enriched population comprises human diploid liver cells.

5. The method of claim 1 in which the liver progenitors are hepatic progenitors, hemopoietic progenitors, mesenchymal progenitors, or mixtures thereof.

6. The method of claim 1 in which the alpha-fetoprotein is full-length alpha-fetoprotein.

8. (Amended). The method of claim 1 in which the debulking step comprises centrifugal elutriation, density gradient centrifugation, countercurrent fluid flow, continuous-flow centrifugation, zonal centrifugation, or combinations thereof.

9. The method of claim 1 which further comprises selective lysis of the mature cells.

11. (Amended) A human hepatic pluripotent progenitor isolated by the method of claim 1.

12. (Twice Amended) A method of preparing a composition comprising an enriched population of human hepatic progenitors comprising:

(a) providing a cell suspension of human liver tissue,

(b) debulking the suspension based on cell size, buoyant density, or a combination thereof to remove mature cells, and

(c) subjecting the debulked suspension to a positive or negative immunoselection, such that a mixture of cells is provided, which mixture of cells is comprised of an enriched population of human liver progenitors, which human liver progenitors themselves, their progeny, or more mature forms thereof exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both.

13. The method of claim 12 in which the liver progenitors are hepatic progenitors, hemopoietic progenitors, mesenchymal progenitors, or combinations thereof.

14. The method of claim 12 in which the immunoselection comprises selecting cells that express markers associated with hemopoietic cells, cells that express markers associated with hepatic cells, cells that express markers associated with mesenchymal cells, or combinations thereof.

15. The method of claim 12 in which the immunoselection comprises selecting from the suspension those cells, which themselves, their progeny, or more mature forms thereof exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both.

16. The method of claim 15 which further comprises selecting those cells which themselves, their progeny, or more mature forms thereof produce full-length alpha-fetoprotein mRNA.

17. The method of claim 12 in which the immunoselection comprises selecting from the suspension those cells that express an adult liver cell-specific marker.

18. The method of claim 12 in which the immunoselection comprises selecting those cells, which themselves, their progeny, or more mature forms thereof express CD14, CD34, CD38, ICAM, CD45, CD117, glycophorin A, connexin 32, osteopontin, bone sialoprotein, collagen I, collagen II, collagen III, collagen IV, or combinations thereof.

19. The method of claim 12 which the immunoselection comprises selecting those cells, which themselves, their progeny, or more mature forms thereof further express alpha-fetoprotein-like immunoreactivity, albumin-like immunoreactivity, or a combination thereof.

20. (Amended) A human hepatic pluripotent progenitor isolated by the method of claim 14.

21. (Twice Amended) A composition comprising an enriched population of human hepatic pluripotent progenitors, their progeny, or more mature forms thereof, which exhibit one or more markers indicative of expression of full-length alpha-fetoprotein, full-length albumin, or both.

22. The composition of claim 21 in which the progenitors comprise hepatic progenitors, hemopoietic progenitors, mesenchymal progenitors, or combinations thereof.

23. The composition of claim 21 in which the progenitors, their progeny, or more mature forms thereof express CD14, CD34, CD38, CD117, ICAM or combinations thereof.

24. The composition of claim 21 in which the progenitors harbor exogenous nucleic acid.

25. The composition of claim 24 in which the exogenous nucleic acid encodes at least one polypeptide of interest.

26. The composition of claim 24 in which the exogenous nucleic acid promotes the expression of at least one polypeptide of interest.

27. (Amended) A method of treating liver dysfunction or disease responsive to treatment with liver progenitors, comprising administering to a subject in need of such treatment an effective amount of human liver progenitors, their progeny, more mature forms thereof, or combinations thereof, in a pharmaceutically acceptable carrier and treating the liver dysfunction or disease.

28. The method of claim 27 in which the human liver progenitors comprises hepatic progenitors, hemopoietic progenitors, mesenchymal progenitors, or combinations thereof.

29. The method of claim 27 further comprising administering simultaneously or sequentially in any order an effective amount of adult human liver progenitors, their progeny, more mature forms thereof, or combinations thereof.

30. The method of claim 27 in which the human liver progenitors are administered parenterally.

31. The method of claim 27 in which the liver disorders or dysfunctions comprise hepatocholangitis, hepatomalacia, hepatomegalia, cirrhosis, fibrosis, hepatitis, acute liver failure, chronic liver failure, cancer, hematologic disorders, hematologic dysfunctions, or inborn errors of metabolism.

32. The method of claim 31 in which the cancer comprises hepatocarcinoma, hepatoblastoma, or both.

33. The method of claim 31 in which the cancer comprises a metastatic tumor in liver deriving from a primary site selected from the group consisting of intestine, prostate, breast, kidney, pancreas, skin, brain, and lung.

34. The method of claim 31 in which the hematologic disorders or dysfunctions include anemia, leukemia, or those induced by chemotherapy, radiation, drugs, viruses, trauma, or combinations thereof.

35. (Amended) A method of treating a disease comprising administering to a subject in need of such treatment an effective amount of human hepatic progenitors, their progeny, or more mature forms thereof in which the human hepatic progenitors, their progeny, or more mature forms harbor exogenous nucleic acid.

38. A cell culture comprising the composition of claim 21, an extracellular matrix component, and a culture medium.

39. A pharmaceutical composition comprising the composition of claim 21 and a pharmaceutically acceptable carrier.

42. (Twice Amended) Isolated human hepatic pluripotent progenitors, their progeny or more mature forms thereof which exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both.

43. (Twice Amended) Isolated human hepatic pluripotent progenitors, their progeny or more mature forms thereof which exhibit the phenotype glycophorin $A^-$, $CD45^-$, alpha-$fetoprotein^{+++}$, $albumin^+$, and $ICAM^+$.

44. The isolated human liver progenitors of claim 43 which further express CD14+, CD34++, CD38++, CD117+, or combinations thereof.

45. (Amended) The method of claim 1 in which the progenitors have a diameter between 5 and 15 microns.

46. (Amended) The method of claim 45 in which the progenitors have a diameter between 8 and 9.4 microns.

47. (New) The method of claim 1 which further comprises selecting those cells which their progeny, or more mature forms thereof exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both.

48. (New) The method of claim 47 in which the selection step comprises panning, affinity chromatography, tagging with fluorescent labels, use of magnetic beads, or combinations thereof.